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In Vitro Cytotoxicity Testing and the Application of Elastic Interconnection Technology for Short-Term Implantable Electronics

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Abstract— **An electronic device was fabricated consisting of 2 flexible electronic circuit islands, interconnected by a 7 cm long elastic interconnection, which could be elongated for at least 50%. This interconnection was based on gold conductor tracks following a 2-D spring pattern, embedded in a biocompatible silicone elastomer. The complete device was embedded in the same silicone elastomer. An in vitro cytotoxicity extraction test, executed on small test-samples in accordance with the ISO 10 993-1 guidelines, revealed that the applied silicone encapsulation to these samples functioned as a good seal for at least 8 days.**

I. INTRODUCTION

Elastic electronics are interesting for certain biomedical applications, where the electronic device should fit in its environment as good as possible. Electronics with a high ability to deform are therefore developed.

Lacour et al. [1] recently demonstrated a technology for elastic electronics, consisting of an elastic polymer substrate, on which stiff subcircuit islands of about 200 x 200 $\mu\mathrm{m}^2$ were interconnected by ultrathin elastic gold tracks. Other work of Lacour et al. [2]–[5] concentrated on the construction and properties of similar ultrathin gold tracks, which are generally achieved by electron-beam evaporating a 20-100 nm thick gold film on an elastic PDMS substrate. Such a way of constructing elastic interconnections is also being studied and optimized by Béfahy et al. [6]. Following Gray et al. (2004) [7], our approach relies on 5 - 70 $\mu\mathrm{m}$ thick metallic tracks, following a 2-D spring pattern and embedded in an elastic polymer material [8]–[14].

This paper describes the construction of an electronic device, which was fully encapsulated in a biomedical grade silicone elastomer film and consisted of 2 flexible circuits, interconnected by a 7 cm long elastic gold interconnection. Giving a first indication about the biocompatibility of such a device, a resistor was soldered on pads containing nickel and gold metal, all of which were embedded in a silicone elastomer film and put through an in vitro cytotoxicity test.

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II. METHOD

The construction of the electronic device was accomplished in three main steps. Firstly, the two flexible electronic circuits and the 7 cm long elastic interconnection were separately fabricated, because a fabrication method in one piece was not possible since the available equipment was not adapted to the large dimensions of the complete device. The flexible circuits were simply implemented on a polyimide flex, without yet embedding it in a silicone film. The elastic interconnection consisted of a 0.25 mm silicone film with conductor material at the bottom. Secondly, the 3 parts were electrically joint together with a conductive paste. The third and last step was encapsulating the complete circuit in a silicone film. For the realization of the elastic interconnection part and the encapsulation of the complete circuit, the used silicone material was Silastic MDX4-4210 from Dow Corning, being a biomedical grade silicone elastomer with a low Young's modulus ($<$ 2MPa) and high elongation (470%).

The 7 cm long interconnection part was constructed following step 1-6 of Fig. 1, which shows an earlier published process flow [9]. In accordance with step 1-4, a gold pattern was plated on a copper-foil using photolithography and gold electrodeposition. After this, the gold interconnection was covered with Dow Corning DA 6524, a stretchable silver-filled silicone elastomer, which should act as a conductive bridge where cracks in the gold track occur due to high stresses. Then, following step 5-6, the substrate was covered with a thin elastic silicone film and the copper-foil was etched, resulting in the elastic interconnection. The pads at both ends of the interconnection were pasted on the pads of the flexible circuits with the conductive silicone paste XCS80091-1 of Emerson & Cuming (Fig. 2). Subsequently, a silicone layer of 1 mm was cast over the complete circuit and cured at 80°C. As illustrated in Fig. 3, the thickness of 1 mm was obtained by moving a blade over a 1 mm thick frame, which surrounded the circuit with the casted viscous silicone on it. Further on, in the same way, a 1 mm thick silicone layer was applied on the backside, which adhered to the earlier applied silicone layer on the front side. Finally, the components were protected by applying an extra 1,5 mm silicone layer on the 2 component areas indicated in Fig. 4. The resulting electronic device is shown in Fig. 5. The left circuits function is to convert a magnetic field generated by an external driver coil into a DC voltage of about 5V, which is the right voltage to drive the array of LED's in the right circuit, on the basis of a microcontroller [13].

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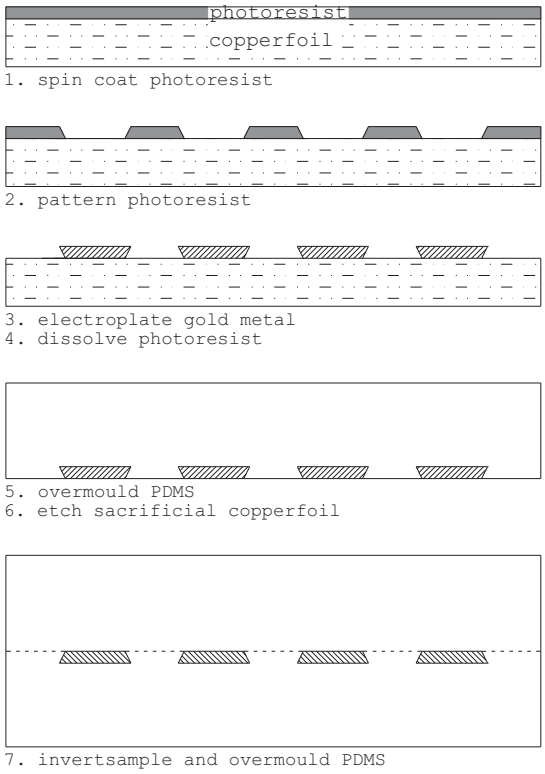


Fig. 1. Process flow to fabricate an elastic interconnection based on pattern plated gold conductor tracks, embedded in an elastic PDMS (polydimethylsiloxane) membrane.

Fig. 2. Two flexible circuit islands were electrically joint by a 7 cm elastic interconnection by gluing the pads of the latter on the pads of the circuits.

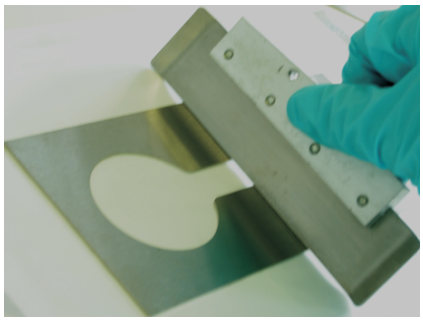


Fig. 3. Before curing an applied viscous silicone layer, the thickness of the layer was brought to 1 mm by moving a blade over a 1 mm frame.

III. EXPERIMENTS

An in vitro toxicity test was performed on small silicone samples of about 1 cm^2, in which a resistor, solder, soldermask and metallic pads of gold and nickel were embedded. Fig. 6 shows a schematic drawing of such a sample. Their production was based on the process scheme of Fig. 1. Metallic pads of 0.5 x 0.6 mm^2 were deposited by executing step 1-4 of Fig. 1, where step 3 consisted of the electrodeposition of about 300 nm Ni, 4 μm Au and 2,2 μm Ni, followed by the electroless deposition of 100 nm Au. The bottom Ni layer was used for easier electroplating gold, the 2,2 μm Ni layer allowed soldering and the 100 nm Au top layer served as oxidation protection layer. Before soldering resistors on these pads, the green solder-resist *Elpemer SD 2463 FLEX-HF* was printed, from which the parts covering the Au-Ni pads were removed by illumination and development. Lead-free solder-paste *Delphine SnAg(3%)Cu(0.5%)* was the used solder material. The soldered resistors, together with the pads and the green soldermask, were embedded into a silicone film by executing step 5-7 of Fig. 1. Before applying the second silicone layer (step 7), an *air-plasma treatment* was done at low pressure ($\approx$ 6 mbar). The resulting sheet, shown in Fig. 7(a), was then cut into single samples of about 1 cm^2 (Fig. 7(b)).

These samples were subjected to a cytotoxicity extraction, to test the presence of possible toxic leaching products

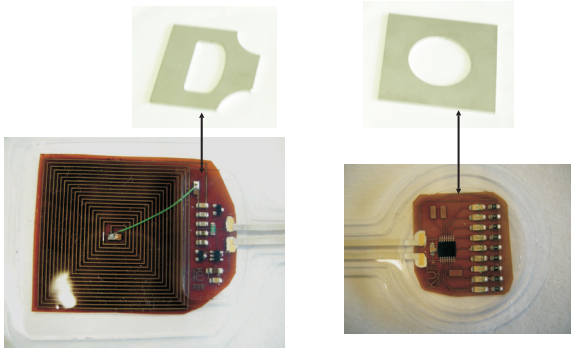
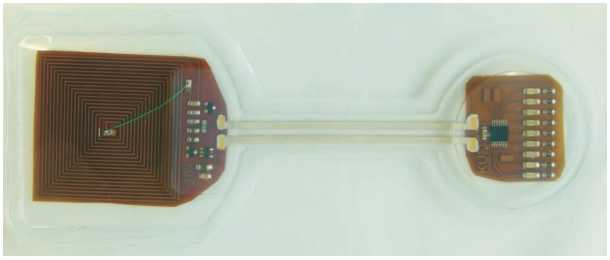
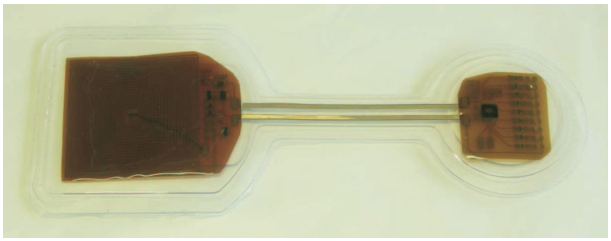


Fig. 4. Two frames of 1.5 mm were used to bring the silicone thickness at the component areas to 2.5 mm.



(a)



(b)

Fig. 5. The resulting flexible electronic device, provided with a 7 cm long elastic interconnection, constructed as a demonstrator within the IWT-SBO Bioflex project. (a) Front side. (b) Backside.

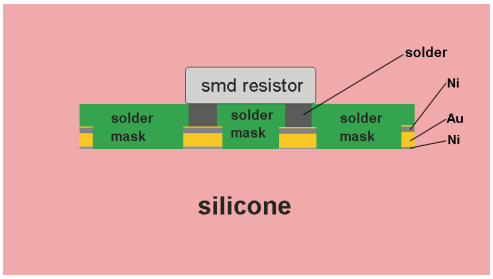
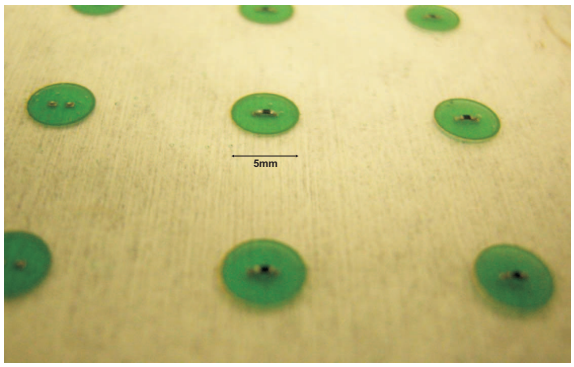
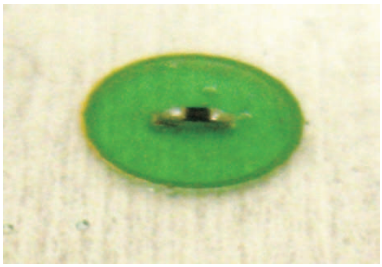


Fig. 6. Schematic drawing of a sample, of which a series was produced and put through an in vitro cytotoxicity extraction test.



(a)



(b)

Fig. 7. (a) Photo of the silicone sheet containing the samples for the in vitro cytotoxicity extraction tests. (b) Single sample.

or metals. At 37°C, 3 samples were incubated in a cell culture medium for 1 and 8 days. This extraction medium was brought onto a cell layer of primary derived chicken embryo fibroblasts. The following day, the cell viability was evaluated using the MTS assay. The reduction of the MTS tetrazolium salt into a soluble formazan product in the mitochondria of viable cells was measured spectrophotometrically ($\lambda = 490$ nm). This absorbance value is proportional to the number of viable cells and can be compared to the absorbance value of control cell culture without contact with the extraction medium. The percentage of viable cells can be determined as the absorbance value of the test cultures divided by the absorbance value of the control cultures, multiplied by 100.

This cytotoxicity test was executed in accordance with the ISO 10 993-1 guidelines and the toxicity of the extraction medium was tested in triplicate.

As additional experiment, the bond strength between 2 silicone layers was determined by measuring the force to peel 2 layers from each other, see Fig. 8. The 2 silicone layers were applied on each other exactly in the same way as during the production of the cytotoxicity samples. The first 1 mm silicone layer was cast on the drum side of a 35 μm plain copper-foil from Circuit Foil Luxembourg. The second layer was cast on the silicone surface that contacted the copper-foil before it was etched. While applying the second silicone layer, an ultra-thin teflon release-foil covered the first layer over a length of 2.5 cm, so that pulling could be started at the non-contacting 2.5 cm ends of the silicone layers, which were cut into stripes of 1 cm wide.

IV. RESULTS

The constructed electronic device functions well and remains functional when stretched in its longitudinal direction. Pulling at both ends of the device forces the 7 cm interconnection to elongate. The device remained functional when elongated 3,5 cm, which corresponds to an elongation of the interconnection of 50%. Higher elongations were not tested in order to not damage the device. Earlier research [9] revealed that similar interconnections could be elongated between 50 and 100%.

In Fig. 9 the mean cell viability percentages of the cells in contact with the extraction media are presented. The standard deviation between the three experiments is indicated by the ”flag” above the bars. The reported viability percentages are around 100%, indicating that there are no toxic leaching products or metals released out of the samples during an 8 day incubation period in an *in vitro* setting.

The result of the peel-test of 4 different samples is shown in Fig. 10. The force necessary to peel the layers from each

Fig. 8. The force was measured to peel the two silicone layers from each other with a pulling machine.

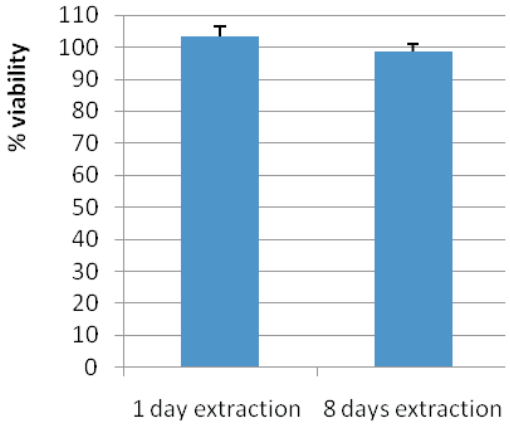


Fig. 9. Cytotoxicity testing. "Samples" are incubated in culture medium for 1 and 8 days. The graph represents the mean cell viability of cell in contact with this medium for 1 day.

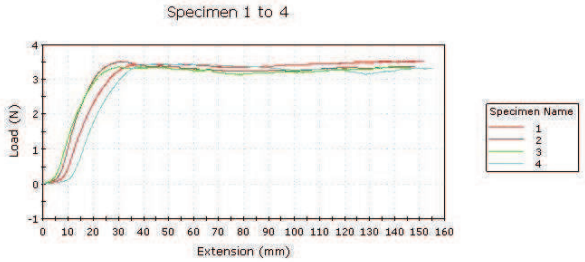


Fig. 10. These graphs display the force to peel two silicone layers from each other in function of the extension. At the rising part of the graphs only an elastic extension of the non-contacting silicone ends occurred. These silicone ends had a cross section of $10\,\mathrm{mm}^2$. The samples peeled at a force of 3.2-3.5 N or a tension in the non-contacting silicone ends of 0.32-0.35 MPa.

other corresponds to the horizontal level of the graphs, being between 3.2 and 3.5 Newton. At the rising part of the graphs only an elastic extension of the non-contacting silicone ends occurred. The silicone layers were 10 mm wide and 1 mm thick. This means the samples started to peel at a tension of up to $0.35\,\frac{N}{\mathrm{mm}^2}$ or $0.35\,\mathrm{MPa}$, which is low. Consequently, little force was necessary to peel the samples by hand.

V. DISCUSSION

A fully flexible electronic device was fabricated, containing a 7 cm long elastic interconnection. The optimalisation of the technology and design of elastic interconnections and flex-stretch transitions are out of the scope of this paper.

The biocompatibility of a similar device highly depends on the sealing capacities of the surrounding silicone, because the electronic circuit itself exposes toxic materials, such as copper and solder. The silicone encapsulation of the cytotoxicity samples proved to be a good seal under the applied in vitro circumstances: no toxic leaching products or metals were present in the extraction medium after 8 days. The low bond strength experienced in the peel test indicates that further research is necessary to improve the chemical bond between the silicone layers. Nevertheless, the adhesion was already good enough to inhibit the transport of toxic entities between the 2 silicone layers during 8 days.

VI. CONCLUSION

A technology has been obtained to combine flexible circuits with elastic interconnections in one electronic device, encapsulated in a silicone elastomer. Aiming the development of short-term implantable flexible and elastic electronic circuits, a preliminary in vitro cytotoxicity test was performed on small silicone samples in which toxic metals and components were embedded. These samples were found to be biocompatible in a cell culture medium for at least 8 days.

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